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Effect of temperature and genetic structure on adaptive evolution at a dynamic range edge in the North American gypsy moth (Lymantria dispar L.)

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Effect of temperature and genetic structure on adaptive evolution at a dynamic range edge in the North American gypsy moth (*Lymantria dispar* L.)

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Abstract

EFFECT OF TEMPERATURE AND GENETIC STRUCTURE ON ADAPTIVE EVOLUTION AT A DYNAMIC RANGE EDGE IN THE NORTH AMERICAN GYPSY MOTH (LYMANTRIA DISPAR L.)
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The study of biological invasions is not only essential to regulate their vast potential for ecological and economical harm, they offer a unique opportunity to study adaptive evolution in the context of recent range expansions into novel environments. The North American invasion of the gypsy moth, *Lymantria dispar* L., since its introduction in 1869 to Massachusetts, has expanded westward to Minnesota, northward to Canada, and southward to North Carolina. Fluctuating range dynamics at the southern invasive edge are heavily influenced by heat exposure over their optimal (supraoptimal) during the larval stage of development. We coupled genomic sequencing with reciprocal transplant and laboratory-rearing experiments to examine the interactions of phenotypic, genetic, and environmental variation under selective supraoptimal regimes. We demonstrate that while there is no evidence to support local adaptation in the fitness-related physiological traits we measured, there are clear genomic patterns of adaptation due to differential survival in higher temperatures. Mapping of loci identified as contributing to local adaptation in a selective environment and those associated with phenotypic variation highlighted that variation in larval development time is partly driven by pleiotropic loci also affecting survival. Overall, I highlight the necessity and inferential power gained through replicating environmental conditions using both phenotypic and genome-wide analyses.

INTRODUCTION

Biological invasions are the introduction of a non-native species, accidental or deliberate, to a novel environment that establishes and expands to cause serious economic and ecological harm. Invasive species are estimated to cost the United States more than $125 billion in damages each year (Pimental *et al.* 2000). The study of biological invasions is not only important for resource managers and conservation biologists in regards to management and eradication efforts, but it also offers a unique opportunity to investigate the influence of adaptive evolution on range dynamics (Sakai *et al.* 2001). Species newly established within novel ecological conditions face many challenges, especially since these environments often drastically differ from those in their native range. This is known as the genetic paradox of invasion, which can be characterized by the following question - if many populations are locally adapted to their native environments then how can introduced individuals establish, expand, and in many cases, outperform individuals from native species in novel environments (Sax & Brown 2000; Allendorf & Lundquist. 2003; Estop *et al.* 2016)? One answer to this paradox is that populations of invasive species become locally adapted to novel environments along the invasion front (Frankham *et al.* 2002; Kawecki 2008).

Genetic diversity is necessary for local adaptation, but several processes associated with invasion often reduce diversity (Sakai *et al.* 2001; Allendorf & Lundquist 2003; Kawecki & Ebert 2004; Dlugosch & Parker 2008a; Keller & Taylor 2008; Bock *et al.* 2015; Halbritter *et al.* 2015). For example, population bottlenecks decrease genetic diversity from native population if the founding population contains only a subsample of the total genetic diversity; thus, decreasing the effective population size (Baker & Stebbins 1965; Nei *et al.* 1975; Dlugosch & Parker 2008a). Conversely, many studies have showed that extreme bottlenecks do not constantly lower

genetic variation across all invasion successes and included several ways in which genetic diversity can be increased (Frankham *et al.* 2002; Barrett & Husband 1990; Kirkpatrick & Jarne 2000; Lee 2002; Bock *et al.* 2015). For example, multiple source introductions may restore the reductions in genetic diversity with admixture from genetically distinct source populations, even leading to higher levels of diversity than the native range. Accumulation of mutations as the population size rapidly increases is also a source of novel genetic diversity produced in populations recovering from demographic bottlenecks (Kolbe *et al.* 2004; Keller & Taylor 2010; Savolainen *et al.* 2013). The rate at which new mutations can arise in a population is equal to the population size (*2N* for diploid organisms) times the mutation rate, but has been shown to having a greater chance to rise to fixation in rapidly growing populations (Otto & Whitlock 1997). For example, *Arapidopsis thaliana* has an estimated mutation rate of $6\ x\ 10^{-9}$ per site/per generation but is conservatively estimates to accumulate 20,000 new mutations expanding from 10 to 1000 individuals over 50 years (Ossowski *et al.* 2010; see Dlugosch *et al.* 2015 Box 1).

Populations situated at range margins are of particular ecological and evolutionary interest, because they occupy environments that are novel relative to the remainder of the geographical range (Antonovics 1976) Expansion into novel environments can be facilitated by adaptive evolution (Kawecki 2008; Halbritter *et al.* 2015), although abundant evidence points towards the role of phenotypic plasticity as also being important (Lande 2009; Chevin *et al.* 2010; Lande 2015). Even though these peripheral populations often exhibit decreased genetic diversity as compared to those in the core of the distribution, presumably due to serial population bottleneck events mimicking the original invasion event, connectivity among the previously established populations and those at the range margin, as well as novel mutations, can effectively increase adaptive potential and a response to selection by restoring genetic diversity upon which

natural selection can act (Nei *et al.* 1975; Otto & Whitlock 1997; Eckert *et al.* 2008; Dlugosch & Parker 2008b). Conceptually, this occurs when alleles, either neutral or slightly deleterious, arise through mutation within populations located at the range core and subsequently move to populations located at the range margin through gene flow or migration of individuals during a single colonization event. These once neutral or slightly deleterious alleles are then advantageous in the novel environment so that selection increases their frequencies within populations now located at the range margin (Kawecki 2008).

Founder effects can exhibit similar patterns in peripheral populations by pushing alleles at low-frequency to high frequency when compared to the core population, thus making it difficult to detect the genetic basis of adaptation (Klopfstein *et al.* 2006; Excoffier & Ray 2008; Barrett & Hoekstra 2011; Savolainen *et al.* 2013). For example, spatial genetic patterns in multiple species of *Anolis* are driven more by non-selective factors due to geographic distance rather than adaptive patterns to novel environments (Wang *et al.* 2012). Thus, studies seeking to link biological invasions with local adaptation need to integrate across three key components during hypothesis testing proposed by Sork *et al.* (2013): (1) environment-phenotype correlations to establish the putative role of natural selection on heritable traits, (2) genotype-phenotype correlations to establish the genetic loci controlling heritable traits that were structured across environments, and (3) genotype-environment correlations to identify genomic regions and mechanisms that respond to different selective pressures.

Classical approaches in quantitative genetics, including common gardens and reciprocal transplant experiments, are informative about the relative influences of genetic and environmental variation on fitness-related traits. They also provide direct tests of local adaptation (Kawecki & Ebert 2004). Such experimental designs are often difficult to establish in non-model

organisms due to the feasibility of rearing pedigreed individuals (i.e. sibling groups from multiple parents) derived from natural populations (Hirschhorn & Daly 2005; Savolainen *et al.* 2013). Other studies have foregone the pedigreed approach for a simpler experimental design and the integration of next-generation sequencing that offer the association of genome wide variation to phenotypes (Fournier-Level *et al.* 2011; Gompert *et al.* 2012; Comeault *et al.* 2014). Advances in sequencing technologies have made it possible to examine genetic markers spread throughout the genome of an organism, even without a reference genome sequence, with the goals of testing evolutionary hypotheses about the relative roles of neutral and adaptive processes within natural populations (Parchman *et al.* 2012; Eckert & Dyer 2012). For example, restriction site associated DNA sequencing (RADseq) has become a standard methodology for population genomic analyses due its increasing number of bioinformatics resources, lack of requirement for prior genomic reference, and ability to genotype a large number of individuals in natural populations in a cost-effective manner (Davey *et al.* 2011). Coupling new genomic resources with classical approaches, even those not optimally designed for traditional quantitative genetic inference, has vastly increased the inferential power of local adaptation in natural populations by providing a way to quantify the relative roles of adaptive and neutral processes in determining observed levels of genetic variation (Stinchcombe & Hoekstra 2008; Barrett & Hoekstra 2011).

We use the North American invasion of the gypsy moth (*Lymantria dispar* L.) to examine adaptive evolution at its southern range margin. The gypsy moth is regarded as one of the most well documented biological invasions in modern times (Doane & McManus 1981). Range dynamics of the southern invasion front are known to vary drastically across West Virginia and Virginia, and it is hypothesized that sublethal effects of prolonged exposure to

supraoptimal temperatures during larval development could explain the variation in spread rates in these areas (Tobin *et al.* 2014). Over the last 20 years, range spread has fluctuated from steady progression of 5.7 km/yr in the Appalachian Mountains to roughly stasis in the Piedmont region to considerable range retraction of 9.7 km/yr in the Coastal Plain region (Tobin *et al.* 2014). Empirical work by Thompson *et al.* (2017) examining the physiological effects of supraoptimal temperature in populations along a latitudinal gradient showed sublethal effects of reductions in pupal size but the southern-most populations were less sensitive to the constant supraoptimal temperatures. Evidence of phenotypic and genetic differentiation among the populations should, therefore, be evident where selective pressures from supraoptimal temperature exposure are the strongest (i.e. within the Coastal Plain).

The known physiological constraints due to variable thermal regimes, as well as the quantified patterns of variable spread rates along the southern range margin, thus make *L. dispar* an ideal model to study adaptive evolution and its effect on biological invasions. We aim to quantify the interplay of genetic, phenotypic, and environmental variation using the aforementioned tripartite framework for the study of local adaptation through a combination of field, laboratory, and next-generation sequencing analyses. We hypothesize that will be able to: (1) provide evidence for local adaptation in range margin population of *L. dispar* due to higher thermal regimes and (2) identify the genomic loci that underlie phenotypic and genetic variation associated with local adaptation within varying selective environments. These analyses will give insights the potential for further range expansion in *L. dispar* and also the genetic architecture of adaptation at range margins.

MATERIALS & METHODS

Study System

The gypsy moth, *L. dispar*, is a univoltine, generalist herbivore that feeds on over 300 host foliage species, preferably on oak and aspen, and has been estimated to cost the United States ~$270 million in damages to forests and eradication expenditures annually (Liebhold *et al.* 1995, Aukema *et al.* 2011). The source of the original introduction in North America occurred in Medford, Massachusetts in 1869 by an amateur entomologist who transported them from France (Elkinton & Liebhold 1990). Since then, the gypsy moth has expanded its range to over 900,000 km^2, ranging from Minnesota in the west and from Canada in the north to North Carolina in the south (Tobin *et al.* 2012). While already extensive, the current range only occupies roughly a third of its potentially suitable host habitat in North America (Morin *et al.* 2005). Northern range expansion is known to be limited by prolonged exposure of overwintering eggs to below-freezing temperatures, but less is known about the thermal physiological limits of range expansion in the south (Logan *et al.* 1991; Bale 1993; Gray 2004).

Population Sampling

In the autumn of 2013, 20 egg masses were collected from the Appalachian Mountains of West Virginia, USA (37.5462°N, 81.2184°W; elevation: 871 m) and from the Great Dismal Swamp in the Coastal Plain of Virginia (36.6350°N, 76.5078°W; elevation: 6.5 m). The Appalachian Mountain (AM) population represents the steadily progressing southern range margin of the West Virginia and Virginia mountains. The coastal plain (CP) population represents the static or retreating southern range margin in the coastal plain region of Virginia (Figure 1). The first generation of these sources were reared indoors in the spring of 2014 with

ambient light and temperature on locally-collected northern red oak (*Quercus rubra* L.) foliage in accordance with phenology specific to Richmond, Virginia, USA. Twenty second-generation egg masses were given 60-80 days to embryonate before being allocated to the two study locations to overwinter. Egg masses from each population were mixed to reduce relatedness with approximately half of the eggs overwintering at each location. The eggs were placed in a petri dish with breathable protective housing and attached to a *Q. rubra* at each site to overwinter in a natural environment.

Reciprocal transplant experiment

In the spring of 2015, a natural reciprocal transplant experiment was conducted between the AM and CP populations. Mountain Lake Biological Station was the study site used as representative of the thermal regimes in Appalachian Mountain region, while University of Richmond was used to represent the coastal plain of Virginia (Figure 2). Mountain Lake Biological Station is located in Giles County, Virginia, USA (37.376347°N, 80.522053°W; elevation: 1184 m). It is in the mountain region of Virginia, 64 km from the collection area of the Appalachian Mountain population. University of Richmond is located in Richmond, Virginia, USA (37.573084°N, 77.542114°W; elevation: 61 m). It is on the western edge of the coastal plain region in Virginia, 139 km from the collection area of the Virginia coastal plain.

Overwintering eggs were removed from the trees and allowed to hatch in synchrony with budburst of *Q. rubra* at each site. Hatched larvae were selected at random to minimize relatedness, and placed into 15 one-liter, unwaxed, paper cups with a plastic lid containing pin holes for air exchange at densities of n = 10 larvae/cup. After 21 days, with most individuals at third instar, larvae were reallocated to 11 7.6-liter plastic buckets and covered with a mesh fabric for each population at densities of n = 10 larvae/bucket. Each cup/bucket contained *Q. rubra*

stems with leaves placed either in a floral water tube rested in a stand-alone Styrofoam holder for cups or one-liter plastic flask for buckets. All larvae were fed fresh foliage every three to four days gathered at the same time from a single tree at each location. Larvae were checked for pupation daily, and fully sclerotized pupae were weighed and stored in paper lined 74-ml plastic cups with snap-on lids containing pin holes for air exchange. Pupae were checked daily for adult emergence with sex and date being recorded. All rearing occurred in a screened and shaded, outdoor insect rearing facility to avoid predation and weathering. Only males will be included in analyses as they are more relevant for dispersal and the phenotypes measured will be pupal mass (Mass), larval development time (LDT: hatching to pupation), and pupal duration (PD: pupation to adult emergence).

Controlled chamber experiment

Natural environmental experiments could have erroneous factors influencing variation, such as: variation in relative humidity, host foliage quality, light, and weather. To ensure that only the physiological effects of solely temperature are quantified, the populations were subjected a controlled chamber experiment in the laboratory. This was done by manipulating historical temperature data at upper and lower thermal regimes in both the Appalachian Mountain and Coastal Plain regions of Virginia (Figure 2). The warmest and coolest years over the past 20 years in both sites were chosen based on the methods of Tobin *et al.* (2014) using the number of hours above optimal gypsy moth developmental rate temperature (28°C) during the period of gypsy moth activity for the year (hatching to adulthood). Temperature data were gathered from nearby weather stations with daily maximum and minimum estimated using the same protocol as Tobin *et al.* (2014). Environmental chambers (Percival Scientific, Inc., Model I22VL, Intellus Connect Software) were programmed to mimic the historical temperature data by

changing each hour on the hour based on the rhythmic sine of the estimated daily maximum and minimum temperatures. Light cycles were also kept true to the historical data by switching on and off to the nearest full hour of sunrise and sunset. The historical years chosen were 1993 for the Appalachian Mountains (MW) for the warmest year and 1997 for the coolest and 2005 for the warmest year in the Coastal Plain region (CC/CW), respectively. A constant temperature of 26°C was added as a treatment to quantify relatively non-stressful conditions under an approximately optimal temperature. Additionally, a climate change scenario of +1.7°C relative to the warmest year in Coastal Plain (C+) was added as an extremely selective treatment. The calendar dates for starting the rearing process within each treatment were calculated using the Gypsy Moth Life Stage model (Gray 2004).

The experiment used individuals from the same egg mix as in the reciprocal transplant experiment that overwintered at Mountain Lake Biological Station, which would be considered a less stressful environment than the University of Richmond. Once hatched, larvae from the same population were randomly allocated to 15 74-ml plastic cups with snap-on lids at a density of n = 10 larvae/cup and placed on a tray in each of the five experimental chambers. Each cup contained an artificial diet (USDA, Hamden Formula Gypsy Moth Diet #F9630B, Bio-Serv, Flemington, NJ) poured to an approximate depth of 1-cm before solidifying. Diet was changed weekly and the positions of trays within the environmental chambers were systematically repositioned and rotated. Survival was noted upon diet change, reallocating individuals from cups 11-15 to cups 1-10 to keep the consistent density of n = 10 larvae/cup. After 21 days, with most individuals at third instar, larvae from cups 1-10 were transferred to 177-ml plastic cups covered with a paper lid, keeping the density at n = 10 larvae/cup, while larvae from the remaining cups were discarded. Sclerotized pupae were weighed, placed in individual, paper

lined, 74-ml plastic cups with snap-on lids containing pin holes for air exchange and remained in the respective environmental chambers through adult emergence. Again, only males will be included in analyses as they are more relevant for dispersal and the phenotypes measured will be pupal mass (Mass), larval development time (LDT: hatching to pupation), and pupal duration (PD: pupation to adult emergence).

Phenotypic data analysis

Separate analyses for the reciprocal transplant and controlled chamber experiment were conducted, as the controlled chamber experiment was used as an explicit test of temperature effects. Only individuals that survived until adulthood and had sufficient genetic data after sequencing and variant calling were included in the phenotypic analysis. Analyses were conducted using a two-way generalized linear mixed effects model including population and site/treatment as fixed effects and rearing cup/bucket as a random effect. Independent statistical analyses were performed for each response variable (Mass, LDT, and PD) using a Satterthwaite approximation for degrees of freedom due to unequal sample sizes based on mortality. Statistical significance was assessed using $\alpha = 0.05$, with no correction for multiple tests. All analysis was conducted using the STATS and LME4 libraries in R version 3.3.1 (R Core Team 2016)

Library preparation and sequencing

Genomic DNA was extracted from each male individual in both reciprocal transplant and controlled chamber experiment using Qiagen DNeasy Blood & Tissue kit following the manufacturer's protocol. Library preparation used barcoding and double digest restriction-site associated DNA sequencing (ddRADseq) following the protocol of Parchman *et al.* (2012) with *EcoR1* and *Mse1* restriction enzymes. Single-end sequencing on the Illumina HiSeq 2500

platform was conducted by Virginia Commonwealth University Nucleic Acid Research Facility (VCU NARF) for a total of four multiplexed lanes (n = 384 moths), where n = 96 moths/lane.

Variant calling and imputation

Multiplexed fastq files were demultiplexed using GBSX (Herten *et al.* 2015), version 1.2, and mapped to a reference contig assembly of 277,541 contigs using Bowtie2 (Langmead & Salzberg 2012), using flags --local --very-sensitive-local. The resulting SAM files were converted to their binary equivalent (BAM), sorted, and indexed using Picard, version 2.5.0 (https://broadinstitute.github.io/picard). Sequence variants were called from resulting BAM files using SAMtools and BCFtools (Li *et al.* 2009), version 1.3.1. The variants were filtered using VCFtools (Danecek *et al.* 2011), such that the only single nucleotide polymorphisms (SNPs) kept were biallelic (--min-alleles=2, --max-alleles=2), present in at least 50% of the samples (--max-missing=0.5), and greater than 100 bp apart if present on the same contig (--thin=100).

Additional filtering using Python removed duplicate samples with the most missing data from the variant data and samples lacking sufficient phenotypic data (n = 8 moths removed). Filtering protocol kept SNPs with minor allele frequency (MAF) of at least 1%, depth across samples (DP) >= 100 or DP < 1500, alternate allele call quality (QUAL) >= 20, and inbreeding coefficient (Fis) > −0.5 or Fis < 0.5. Filtering left a dataset of 26,260 SNPs across 376 moths covering 23,180 contigs. Finally, SNPs were oriented according to dosage of globally minor allele.

To account for uncertainty within variant calling, weighted genotypes were calculated by converting Phred-scaled likelihoods in the VCF file to weights of the 0, 1, and 2 genotype calls, *sum*(*weight x genotype*), for a single estimate on a continuous scale from 0 to 2. Considering our low threshold for percentage of samples with missing data (50%), a custom imputation protocol

was also implemented. A within population allele frequency was estimated for each SNP then assumed Hardy-Weinberg equilibrium proportions assigned as the weighed genotype for individuals with missing data at a particular SNP. Again, SNPs were oriented based on global minor allele. This weighted genotype dataset with no missing data was used for the genome-wide association and the allele frequency change analyses.

Genome-wide association study

A genome-wide association study (GWAS) was conducted to identify loci that determine phenotypic variation for Mass, PD, and LDT using a univariate framework implemented in program GEMMA (Zhou *et al.* 2013). Independent linear mixed models constructed for each phenotype in the form:

$$\mathbf{y} = \mathbf{W}\boldsymbol{\alpha} + \mathbf{x}\boldsymbol{\beta} + \mathbf{u} + \boldsymbol{\varepsilon};$$

where **y** is a normal-quantile transformed phenotype, **W** is a matrix of covariates including a column of 1s, **α** is a vector of the corresponding coefficients, **x** is a weighed genotype dataset with no missing data, **β** is an effect size of the locus, **u** is a vector of random effects (including **K**, which is a relatedness matrix), and **ε** is a vector of errors. A Wald's test was used to determine statistical significance for each SNP, where the test compares likelihoods in linear mixed models with and without effects of a SNP. Multiple test corrections were implemented using method of Storey and Tibshirani (2003), which is based on FDR *Q*-values. A threshold of $Q < 0.2$ was used to determine statistical significance (e.g. Lamara *et al.* 2016; Hallingbäck *et al.* 2016).

After identifying the SNPs significantly correlated to one of the three phenotypes, the annotations for the contig containing each SNP were pulled from the reference contig assembly. Annotations derive from analysis of the reference contig assembly using MAKER (Campbell *et*

al. 2014), version 2.31.9, using Augustus (Stanke & Waack 2003) gene predictions on *Heliconius melpomeme* genome as the species model. Further exploration of annotations used BLASTN (Zhang *et al.* 2000) version 2.6.1+, to identify putative homologs in other Lepidopteran species.

Genetic variation and allele frequency change

Population structure, including structure among treatments, was assessed using estimates of multilocus F_{ST} from HIERFSTAT (Goudet & Jombart 2015), version 0.04-22, library in R and a bootstrapped (n = 1000) 95% confidence interval was estimated in a custom Python script. A principal components analysis (PCA) was conducted for all individuals within the reciprocal transplant experiment following Patterson *et al.* (2006). All genotypes were centered and standardized prior to analysis. The top ten PC axes were selected for an analysis of variance (ANOVA) to test for a significant effect of site, population, and site by population interaction with a $\alpha = 0.05$ threshold. These analyzes were conducted in a multivariate framework with all 26,262 SNPs included; it is highly unlikely that all loci are contributing to local adaptation under the temperature regimes.

To specifically target loci that may be contributing to local adaptation, allele frequencies were estimated within each experimental site/treatment and population for each locus on our imputed weighed genotype SNP dataset. We estimated allele frequency change (Δaf) within a population between the two sites in the reciprocal transplant, using AM as the null allele frequency or non-selective environment (AM-CP), and kept the tail 0.5% loci in each direction for a total of 262 most differentiated loci. Due to selective pressures that may exist nature not related to temperature, we compared tail loci between the reciprocal transplant and the controlled chamber experiment to test if these loci had constantly the greatest Δaf and show solely

temperature was the driver. Estimated Δaf used the constant 26°C chamber as the null allele frequency or non-selective treatment to compare to the four selective treatments (26°C-MW/CC/CW/C+). Again, taking the tail 0.5% in each direction, we identified the loci that were the most differentiated in the reciprocal transplant and at least one of the treatments in the controlled chamber experiment.

Further analyses were conducted to test: (1) Are there more shared SNPs in the tails between environments than we would expect by chance? (2) Does the direction of allele frequency change among the tails stay the same across the environments? We generated a null model by randomly assigned individuals treatments (1000 times) within the same population, estimated allele frequencies, and conducted the same analysis as above to identify the number commonly shared loci between the reciprocal transplant and controlled chamber experiment. The null model was compared to the number of shared loci to distinguish whether this was greater than expected by chance. Also, we examined the directionality of Δaf in the shared loci by creating a contingency table and testing for significance with a Chi-squared test and a $\alpha = 0.05$ threshold.

RESULTS

Reciprocal transplant experiment

Site had a significant effect on all phenotypic traits regardless of population (Mass: $F_{1,166}$ =139.4, $P < 0.0001$; LDT: $F_{1,160}$ =157.4, $P < 0.0001$; PD: $F_{1,167}$ =1249, $P < 0.0001$). For both populations, individuals at the warmer CP site had significantly reduced Mass (20.8%), LDT (9.8%) and PD (29.1%) compared to the cooler AM site (Figure 3, summary statistics see Table S1). Exploring LDT more closing due to the quantitative differences among populations within

the CP site, a *post-hoc* analysis detected a population effect near the significance threshold ($F_{1,98}$ =3.40, P = 0.0682). Overall there was no significant effect of population or site by population interaction in the phenotypes measured, indicating a lack of evidence for local adaptation in the two populations and the three phenotypes that were measured (Table 1).

Controlled chamber experiment

The controlled chamber experiment results followed similar patterns as the natural reciprocal transplant with LDT ($F_{4,195}$ =515.5, P < 0.0001) and PD ($F_{4,194}$ =38.88, P < 0.0001) having a significant effect of treatment but no effect of population or population by treatment interaction. Pupal mass showed no significant effect of treatment, population, or their interaction (Table 1). While there may be some interpretation of the physiological implications of the quantitative differences among the phenotypes, this again exhibits no phenotypic evidence of adaptive divergence among the two populations due to temperature (Figure 4, summary statistics see Table S1).

Genome-wide association study

Only a small fraction of SNPs had statistically significant effects on measured phenotypes (Mass: 0, LDT: 32, PD: 3), but the proportion of phenotypic variance explained by genotype (PVE) is relevant (Mass: [17.53-48.74%], LDT: [5.85-31.01%], PD: [23.10-52.39%]). The effect size of the locus (β) ranged from: Mass = [-2.011, 2.047], LDT = [-1.807, 1.971], PD = [-1.998, 2.074]; which is in units of standard deviations of normal quantile transformed data. Descriptive summary statistics, including the raw effect sizes of the locus, were calculated for the significantly associated loci (Table 2). Annotations were pulled from the BLAST hits to find that out of the 35 loci, 83% (known function in 54%) of the contigs were found to have sequence

similarity with a gene, protein, or mRNA in another Lepidopteran species (Figure 5; see supplemental T2 for full account of accession number, percent identity, E-value, and bit-score).

Genetic variation and allele frequency change

Global multilocus F_{ST} was estimated to be 0.0499 with a 95% confidence interval of 0.0486 - 0.0511 (Figure S1). Genetic structuring among the populations became apparent with the PCA, showing distinct clustering on the first two PCs, explaining 8.07% of the total variance (Figure 6). The three distinct clusters of the AM population suggested sub-structuring within the population. Opposed to the phenotypic analyses, the first 10 genetic PCs showed a significant interaction of site by population effect ($F_{10,158}$ =1.964, P = 0.0406).

We found many loci that differentiated between sites/treatments within the reciprocal transplant (Δaf = [-0.414,0.397]) and controlled chamber experiment (Δaf = [-0.460, 0.668]). we identified 107 shared loci (82 unique loci) that were the most differentiated in the reciprocal transplant and at least one of the treatments in the controlled chamber experiment. A total of 107 shared outliers was unexpected by chance ($P < 0.0001$) and the directionality of Δaf within the shared loci stayed consistent across the experiments ($\chi^2_{df=1} = 57.99$, $P < 0.0001$; Figure 7). By showing that the loci were repeated across the experiments and the directionality of the Δaf stayed consistent.

The GWAS significantly associated 35 loci to the phenotypic measurements and the Δaf analysis identified 107 shared loci that are likely contributing to local adaptation to higher temperatures. Overlap in the two sets of loci was examined with the expectation that little to none would be found considering the phenotypes measured appear to not locally adapted to the

environments tested. A total of 10 unique loci were overlapping in both analyses with nine of the loci associated to LDT (Table 3).

DISCUSSION

This experiment identified loci that contribute to local adaptation in range edge populations of *L. dispar* as a response to high temperature exposure. This finding is consistent with our hypotheses that the selective pressures of heat in the Coastal Plain are driving adaptation. Evidence of local adaptation, however, is not apparent in the three phenotypes measured. While these phenotypes are temperature responsive and genetically determined, including evidence of local adaptation in range-wide samples (Friedline *et al., unpublished data*), they appear not to affect fitness directly in our experiments.

Reciprocal transplant experiments provide evidence for local adaptation if local populations have a higher fitness than non-local populations when examined in their home environments (Kawecki & Ebert 2004). While measured phenotypes are often clearly components of fitness, they often vary in the way and magnitude of their effects on fitness itself. Thus, the choice of phenotype to measure is crucial to tests of local adaptation (Sork *et al.* 2013), as focus on a conditionally unimportant phenotype to fitness in the experimental environments can lead to false inference. Genome-wide scans for changes in allele frequencies across treatments or through time within experimental studies, where environmental conditions are directly manipulated to test physiologically based hypotheses, may provide a less biased way to identify the loci contributing to fitness differences (Barrett & Hoekstra 2011; Nosil *et al.* 2009). This is because changes to the frequencies of alleles at loci within the genome are directly documented across treatments, rather than through measurements of phenotypes that may be unrelated to fitness in the experimental treatments. Under this framework, once confounding

issues have been quantified and accounted for, large changes to allele frequencies at a locus imply that this locus affects fitness (Nielsen 2005; Barrett & Hoekstra 2011). Similar approaches have been used to identify genomic regions responsive to selective regime in both plants and insects (Porcher *et al.* 2006; Burke *et al.* 2010; Turner *et al.* 2011; Zhou *et al.* 2011; Gompert *et al.* 2014), but only a few studies have compared results across natural and laboratory environments (Michel *et al.* 2010; Ledoux *et al.* 2015). Of these, Michel *et al.* (2010) were the only investigators to successfully show that the focal trait was responsive to the hypothesized selective pressure; demonstrating that most (16 out of 17) of the genomic regions to be differentiated under natural host-differences to also be replicated in the laboratory selective experiment targeting adult ecolsion time in *Rhagoletis pomonella* W.

We determined that populations were locally adapted to higher temperatures, which was driven by differential survival. By only phenotyping and sequencing the individuals that survived until adult emergence, we are implicitly measuring the effect of survival among environments. Observed allele frequency shifts are thus driven by individuals with a genetic disposition to survive in the higher thermal regimes. Loci that were the most differentiated were replicated in both the reciprocal transplant and the controlled chamber experiments, thus providing a consistent conclusion that these loci contribute to variation in survival as a function of temperature. While non-selective processes such as drift could influence large allele frequency shifts, it is highly unlikely that they would be replicated across multiple experimental environments in the same direction of change (Luikart *et al.* 2003).

Poikilothermic organisms, like *L. dispar*, have been shown to experience fluctuations in development rate, mass, fecundity, and survival as a direct response to temperature, typically a negative one when exposed to supraoptimal temperatures (Logan *et al.* 1991; Kingsolver &

Woods 1997; Thompson *et al.* 2017). The three phenotypes we chose to measure (Mass, LDT, and PD) have commonly been used as proxies for fitness in *L. dispar*. Heritability estimates for these traits range from 0.314 - 0.479 for Mass, 0.584 – 0.703 for LDT, and 0.181 – 0.357 for PD, but have typically been measured in host variability experiments (Lazarević *et al.* 1998; Lazarević *et al.* 2002; Lazarević *et al.* 2007; Lazarević *et al.* 2008; Janković-Tomanić & Lazarević 2012; Páez *et al.* 2015). Additionally, pupal mass can be calculated as a reproductive index of fecundity (Capinera & Barbosa 1977) as female size is directly correlated to egg load and also, variation between male and female development times can cause non-overlapping emergence times and hinder mating success (Cotarini *et al.* 2009; Tobin *et al.* 2009).

Genome-wide association studies can provide insights to the genetic architecture of fitness-related traits (Barrett & Hoekstra 2011; Comeault *et al.* 2014). We identified loci in a univariate framework determined to underlie the variation in the phenotypes, even after a FDR multiple test correction was applied. Associated loci encode proteins that often made biological sense when extrapolating their function from homologs in other insects. For example, a fibroin gene in *Antheraea yamamai* G., which is shown to be involved in silk production, an important component in making protective tunnels and pupation casings; highly conserved across all Lepidopterans; differentially expressed and under selection (Craig & Riekel 2002; Collin *et al.* 2010).

The expectation for the comparison of loci from the GWAS and Δaf analyses was to show little to no overlap. This was because the phenotypes measured exhibited patterns inconsistent with local adaptation, while there was strong evidence for local adaptation in the genomic data relative to the environments tested. Interestingly, nine of the 10 overlapping loci were associated to LDT, and LDT was the only trait that had a marginally evident effect of

population or site by population in the phenotypic analyses. This effect occurred solely in the CP site, which is expected to experience the greatest selective pressure based on its documented patterns of recent retraction. It is likely that the variation in LDT within the CP site is thus being driven by a pleiotropic effect due to selection upon survival. Survival is more closely related to fitness and thus it is likely the main driver of local adaptation in this scenario. Gompert *et al.* (2014) showed similar architectural effects in the natural populations of *Timema cristinae* V. when examining allele frequency change due to host-related selection on a known adaptive color phenotype. They demonstrated phenotypic divergence in the selective sites, as well as loci under selection associated with this phenotype, but found no evidence that the most differentiated loci between the sites were associated with the phenotype, thus exhibiting a discordance of patterns of selection and fitness effects as shown here.

Examining adaptive evolution at the dynamic range margin of *L. dispar* offers insights to the interplay on phenotypic, genetic, and environmental variation and the processes that facilitate their interactions. This is the first study, to our knowledge, in a non-model species that makes use of field, laboratory, and genomic experiments to observe adaptation at a range margin. While we found no evidence of adaptation for the commonly measured insect phenotypes, our genome-wide analyses showed adaptive divergence for allele frequencies at loci correlated to survival in higher thermal regimes. The identified loci make biological sense and thus warrant further functional investigation. The evidence of selective pressures on survival driving variation in LDT also highlights the importance of pleiotropy on genetic architecture. This effect would not have been evident if the combination of next-generation sequencing and classical quantitative genetic approaches, such as reciprocal transplants that impose selection in a relevant context, was not investigated

The progression of *L. dispar* invasion across North America has been extremely well-documented and under constant management through pheromone-baited trapping implemented by the Slow the Spread program since 1999 (Tobin & Blackburn 2007). This study exemplifies the need for direct analyses of how adaptive evolution at the southern range margin in *L. dispar* might impact future spread rates. As there is no current geographical barrier limiting the range in the south, it must be due to biotic or abiotic factors. If high temperatures are in fact limiting spread rates and selection drives to adaptation in range edge populations to become tolerate to this heat stress, it could potentially drive range expansion in the southern invasion front. This could impact the Slow the Spread program's spread rate patterns predictions and areas of focus for management and eradication efforts.

LITERATURE CITED

Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. *Conservation Biology*, 17, 24–30

Antonovics J (1976) The nature of limits to natural selection. *Annuals of Missouri Botanical Gardens*, 63, 224–247

Baker HG, Stebbins GL (1965) *The Genetics of Colonizing Species*. Academic Press, New York.

Bale JS (1993) Classes of insect cold hardiness. *Functional Ecology*, 7, 751-753

Barrett RDH, Hoekstra HE (2011) Molecular spandrels: tests of adaptation at the genetic level. *Nature Reviews Genetics*, 12, 767–780

Barrett SCH, Husband BC (1990) The genetics of plant migration and colonization. In: *Plant Population Genetics, Breeding, and Genetic Resources* (eds Brown AHD, Clegg MT, Kahler AL, Weir BS), 254–277. Sinauer & Associates, Sunderland, Massachusetts.

Bierne N, Roze D, Welch JJ (2013) Pervasive selection or is it...? Why are FST outliers sometimes so frequent? *Molecular Ecology*, 22, 2061–2064.

Bock DG, Caseys C, Cousens RD, Hahn MA, Heredia SM, Hübner S, Turner KG, Whitney KD, Rieseberg LH (2015) What we still don't know about invasion genetics. *Molecular Ecology*, 24, 2277–2297 doi:10.1111/mec.13032

Burke MK, Dunham JP, Shahrestani P, Thornton KR, Rose MR, Long AD (2010) Genome-wide analysis of a long-term evolution experiment with Drosophila. *Nature*, 467, 587–590

Cantarel BL, Korf I, Robb SMC, Parra G, Ross E, Moore B, Holt C, Sánchez-Alvarado A, Yandell M (2008) MAKER: An easy-to-use annotation pipeline designed for emerging model organism genomes. *Genome Research*, 18, 188–196

Capinera JL, Barbosa P (1977) Influence of natural diets and larval density on gypsy moth, *Lymantria dispar* (Lepidoptera: Orgyiidae) egg mass characteristics. *Canadian Entomologist*, 12, 1313–1318

Chevin LM, Lande R, Mace GM (2010) Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biology*, 8, e1000357

Collin MA, Mita K, Sehnal F, Hayashi CY (2010) Molecular evolution of lepidopteran silk proteins: insights from the ghost moth, *Hepialus californicus*. *Journal of Molecular Evolution*, 70(5), 519-529

Comeault AA, Soria-Carrasco V, Gompert Z, Farkas TE, Buerkle AC, Parchman TL, Nosil P (2014) Genome-wide association mapping of phenotypic traits subject to a range of intensities of natural selection in Timema cristinae. *The American Naturalist*, 183, 711–727

Contarini M, Onufrieva KS, Thorpe KW, Raffa KF, Tobin PC (2009) Mate-finding failure as an important cause of Allee effects along the leading edge of an invading insect population. *Entomologia Experimentalis et Applicata*, 133, 307–314.

Cooper TF, Rosen DE, Lenski RE *(2003)* Parallel changes in qene expression after 20,000 generations of evolution in *Escherichia coli. Proceedings of the National Academy of Sciences*, 100, 1072-1077

Cooper VS, Lenski RE (2000) The population genetics of ecological specialization in evolving *Escherichia coli* populations. *Nature*, 407, 736-739

Craig CL, Riekel C (2003) Comparative architecture of silks, fibrous proteins and their encoding genes in insects and spiders. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 135(4), 721-722

Danecek P, Auton A, Abecasis G et al. (2011) The variant call format and VCFtools. *Bioinformatics*, 27, 2156–2158

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics*, 12, 499–510

Dlugosch KM, Anderson SR, Braasch J, Cang FA, Gillette H D (2015) The devil is in the details: genetic variation in introduced populations and its contributions to invasion. *Molecular Ecology*, 24, 2095–2111 doi:10.1111/mec.13183

Dlugosch KLM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, 17, 431–449.

Dlugosch KM, Parker IM (2008) Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecology Letters*, 11, 701–709 doi:10.1111/j.1461-0248.2008.01181.x

Doane CC, McManus ML (eds) (1981) *The Gypsy Moth: Research Toward Integrated Pest Management*. USDA-Forest Service Technical Bulletin 1584, Washington, DC

Eckert AJ, Dyer RJ (2012) Defining the landscape of adaptive genetic diversity. *Molecular Ecology*, 21(12), 2836–2838 doi:10.1111/j.1365-294X.2012.05615.x

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Molecular Ecology*, 17, 1170–1188 doi:10.1111/j.1365-294X.2007.03659.x

Estoup A, Ravigné V, Hufbauer RA, Vitalis R, Gautier M, Facon B (2016) Is there a genetic paradox of biological invasion? *Annual Reviews of Ecology, Evolution, and Systematics*, 47, 51–72

Excoffier L, Ray N (2008) Surfing during population expansions promotes genetic revolutions and structuration. *Trends in Ecology and Evolution*, 23, 347–351

Fourcade Y, Chaput-Bardy A, Secondi J, Fleurant C, Lemaire C (2013) Is local selection so widespread in river organisms? Fractal geometry of river networks leads to high bias in outlier detection. *Molecular Ecology* 22, 2065–2073

Fournier-Level A, Korte A, Cooper MD, Nordborg N, Schmitt J, Wilczek AM (2011) A map of local adaptation in *Arabidopsis thaliana*. *Science*, 334, 86–89

Frankham R (2005) Invasion biology: resolving the genetic paradox in invasive species. *Heredity*, 94, 385–385

Gray DR (2004) The gypsy moth life stage model: landscape-wide estimates of gypsy moth establishment using a multi-generational phenology model. *Ecological Modelling*, 176, 155–171

Gompert Z, Lucas LK, Nice CC, Buerkle AC (2012) Genome divergence and the genetic architecture of barriers to gene flow between *Lycaeides idas* and *L. melissa*. *Evolution*, 67, 2498–2514

Gompert Z, Comeault AA, Farkas TE, Feder JL, Parchman TL, Buerkle AC, Nosil P (2014) Experimental evidence for ecological selection on genome variation in the wild. *Ecology Letters*, 17, 369–379

Goudet J, Jombart T (2015) hierfstat: estimation and tests of hierarchical F-statistics. *R package version 0.04-22* https://CRAN.R-project.org/package=hierfstat

Halbritter, AH, Billeter R, Edwards PJ, Alexander JM, (2015) Local adaptation at range edges: comparing elevation and latitudinal gradients. *Journal of Evolutionary Biology*, 28, 1849–1860 doi:10.1111/jeb.12701

Hallingbäck HR, Fogelqvist J, Powers SJ *et al.* (2016) Association mapping in *Salix viminalis* L. (Salicaceae) – identification of candidate genes associated with growth and phenology. *Global Change Biology: Bioenergy*, 8, 670–685 doi:10.1111/gcbb.12280

Handley LJL, Estoup A, Evans DM, Thomas CE, Lombaert E, Facon B, Aebi A, Roy HE (2011) Ecological genetics of invasive alien species. *Biocontrol*, 56, 409–428

Herten K, Hestand MS, Vermeesch JR, Houdt JKV (2015) GBSX: a toolkit for experimental design and demultiplexing genotyping by sequencing experiments. *BMC Bioinformatics*, 16, 73

Huang, H, Knowles LL (2016) Unforeseen Consequences of Excluding Missing Data from Next-Generation Sequences: Simulation Study of RAD Sequences. *Systematic Biology*, 17, 369–379 doi: 10.1093/sysbio/syu046

Hirschhorn JN, Daly MJ (2005) Genome-wide association studies for common diseases and complex traits. *Nature Reviews Genetics*, 6, 95–108

Janković-Tomanić M, Lazarević J (2012) Effects of temperature and dietary nitrogen on genetic variation and covariation in gypsy moth larvae performance traits. *Archives of Biological Science, Belgrade*, 64, 1109–1116

Kawecki TJ (2008) Adaptation to marginal habitats. *Annual Review of Ecology, Evolution, and Systematics*, 39(1), 321-342

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters*, 7, 1225–1241. doi:10.1111/j.1461-0248.2004.00684.x

Keller SR, Taylor DR (2008) History, chance, and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. *Ecology Letters*, 8, 852–56

Keller SR, Taylor DR (2010) Genomic admixture increases fitness during a biological invasion. *Journal of Evolutionary Biology*, 23, 1720–1731

Kingsolver JG, Woods HA (1997) Thermal sensitivity of growth and feeding in Manduca sexta caterpillars. *Physiological Zoology*, 70, 631–638

Kirkpatrick M, Barton NH (1997) Evolution of a species' range. *American Naturalist*, 150, 1–23

Kirkpatrick M, Jarne P (2000) The effects of a bottleneck on inbreeding depression and the genetic load. *American Naturalist*, 155, 154–167

Klopfstein S, Currat M, Excoffier L (2006) The fate of mutations surfing on the wave of a range expansion. *Molecular Biology and Evolution*, 23, 482–490

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution*, 16, 199–204

Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, 431, 177–81

Kremer A, Le Corre V (2011) Decoupling of differentiation between traits and their underlying genes in response to divergent selection. *Heredity*, 108, 375–385

Lamara M, Raherison E, Lenz P, Beaulieu J, Bousquet J, MacKay J (2016) Genetic architecture of wood properties based on association analysis and co-expression networks in white spruce. *New Phytologist*, 210, 240–255 doi:10.1111/nph.13762

Lande R (2009) Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology*, 22, 1435–1446

Lande R (2015) Evolution of phenotypic plasticity in colonizing species. *Molecular Ecology*, 24, 2038–2045

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nature methods*, 9, 357–359.

Lazarević J, Perić-Mataruga V, Ivanović J, Anđelković M (1998) Host plant effects on the genetic variation and correlations in the individual performance of the gypsy moth, *Functional Ecology*, 12, 141–148 doi: 10.1046/j.1365-2435.1998.00166.x

Lazarević J, Perić-Mataruga V, Stojković B, Tucić N (2002) Adaptation of the gypsy moth to an unsuitable host plant, *Entomologia Experimentalis et Applicata*, 102, 75–86 doi: 10.1046/j.1570-7458.2002.00926.x

Lazarević J, Peric-Mataruga V, Tucić N (2007) Pre-adult development and longevity in natural populations of Lymantria dispar (Lepidoptera: Lymantriidae). *European Journal of Entomology*, 104(2), 211-216 doi: 10.14411/eje.2007.033

Lazarević J, Nenadović V, Janković-Tomanić M, Milanović S (2008) Genetic variation and correlations of life-history traits in gypsy moths (Lymantria dispar L.) from two populations in Serbia. *Archives of Biological Science, Belgrade*, 60, 619-627

Li H, Handsaker B, Wysoker A, *et al.* (2009) The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25, 2078–2079

Le Corre V, Kremer A (2003) Genetic variability at neutral markers, quantitative trait land trait in a subdivided population under selection. *Genetics*, 164, 1205–1219

Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, 17, 386–391

Liebhold AM, Elkinton JS, Zhou C, *et al.* (1995) Regional Correlation of Gypsy Moth (Lepidoptera: Lymantriidae) Defoliation with Counts of Egg Masses, Pupae, and Male Moths. *Environmental Entomology*, 24, 193–203

Liebhold A, Mastro V, Schaefer PW (1989) Learning from the Legacy of Leopold Trouvelot. *Bulletin of the Entomological Society of America*, 35, 20–22

Logan JA, Casagrande RA, Liebhold AM (1991) Modeling environment for simulation of gypsy moth (Lepidoptera: Lymantriidae) larval phenology. *Environmental Entomology*, 20, 1516-1525

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, 4, 981–994

Lynch M, Walsh B (eds) (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Inc., Sunderland, MA

McKay JK, Latta RG (2002) Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution*, 17, 285–291

Morin RS, Liebhold AM, Luzader ER, Lister AJ, Gotts-chalk KW, Twardus DB (2005) Mapping host-species abundance of three major exotic forest pests. *USDA Forest Service Research Paper NE-726*, Newtown Square, PA.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, 29, 1–20

Nielsen R (2005) Molecular signatures of natural selection. *Annual Review of Genetics*, 39, 197–218

Neiman M, Linksvayer TA (2006) The conversion of variance and the evolutionary potential of restricted recombination. *Heredity*, 96, 111–121

Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*, 18, 375–402 doi:10.1111/j.1365-294X.2008.03946.x

Ossowski S, Schneeberger K, Lucas-Lledo JI *et al.* (2010) The rate and molecular spectrum of spontaneous mutations in Arabidopsis thaliana. *Science*, 327, 92–94

Páez DJ, Fleming-Davies AE, Dwyer G (2015) Effects of pathogen exposure on life-history variation in the gypsy moth (*Lymantria dispar*). *Journal of Evolutionary Biology.*, 28, 1828–1839 doi:10.1111/jeb.12699

Pannell JR, Charlesworth B (2000) Effects of metapopulation processes on measures of genetic diversity. *Philosophical Transaction sof the Royal Society of London Series B: Biological Sciences*, 355, 1851–1864

Parchman TL, Gompert Z, Mudge J, *et al.* (2012) Genome-wide association genetics of an adaptive trait in lodgepole pine. *Molecular Ecology*, 21, 2991–3005

Patterson N, Price AL, Reich D (2006) Population structure and eigenanalysis. *PLoS genetics*, 2, e190.

Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. *BioScience*, 50, 53–65

Porcher EL (2006) Genetic differentiation of neutral markers and quantitative traits in predominantly selfing metapopulations: confronting theory and experiments with *Arabidopsis thaliana*. *Genetics Research: Cambridge*, 87, 1-12

R Core Team (2016) R: A language and environment for statistical computing. Vienna, Austria

Sakai AK, Allendorf FW, Holt JS *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, 32(1), 305-332

Santure AW, Wang J (2009). The joint effects of selection and dominance on the Qst –Fst contrast. *Genetics*, 181, 259–276.

Savolainen O, Outi S, Martin L, Juha M (2013) Ecological genomics of local adaptation. Nature reviews. *Genetics*, 14, 807–820

Sax DF, Brown JH (2000) The paradox of invasion. *Global Ecology and Biogeography*, 9, 363–371

Simberloff D (2009) The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, 40, 81–102

Stanke M, Waack S (2003) Gene prediction with a hidden Markov model and a new intron submodel. *Bioinformatics* , 19supp(2), ii215–25.

Storey JD, Tibshirani R (2003) Statistical significance for genome-wide studies. *Proceedings of the National Academy of Sciences*, 100, 9440–9445

Tobin PC, Bai BB, Eggen DA, Leonard DS (2012) The ecology, geopolitics, and economics of managing *Lymantria dispar* in the United States. *International Journal of Pest Management*, 58, 195–210

Tobin, P.C. & Blackburn, L.M. (eds). (2007) *Slow the Spread: A National Program to Manage the Gypsy Moth*. USDA Forest Service Northern Research Station General Technical Report NRS-6, Newtown Square, PA.

Tobin PC, Gray DR, Liebhold AM (2014) Supraoptimal temperatures influence the range dynamics of a non-native insect. *Diversity and Distributions*, 20, 813–823

Tobin PC, Robinet C, Johnson DM, Whitmire SL, Bjørnstad ON, Liebhold AM (2009) The role of Allee effects in gypsy moth, *Lymantria dispar* (L.), invasions. *Population Ecology*, 51, 333–384

Thompson LM, Faske TM, Banahene N, Grim D, Agosta SJ, Parry D, Tobin PC, Johnson DM, Grayson KL (2017) Variation in growth and developmental responses to supraoptimal temperatures near latitudinal range limits of gypsy moth *Lymantria dispar* (L.), an expanding invasive species. *Physiological Entomologyl*, 42, 181–190, doi:10.1111/phen.12190

Turner TL, Stewart AD, Fields AT, Rice WR, Tarone AM (2011) Population-based resequencing of experimentally evolved populations reveals the genetic basis of body size variation in Drosophila melanogaster. *PLoS Genetics*, 7, e1001336. doi:10.1371/journal.pgen.1001336

Wang IJ, Glor RE, Losos JB (2012) Quantifying the roles of ecology and geography in spatial genetic divergence. *Ecology Letters*,16, 175–182

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*, 7(1-2), 203-214

Zhou D, Udpa N, Gersten M, *et al.* (2011) Experimental selection of hypoxia-tolerant Drosophila melanogaster. *Proceedings of the National Academy of Sciences*, 108, 2349–2354

Zhou X, Carbonetto P, Stephens M (2013) Polygenic modeling with Bayesian sparse linear mixed models. *PLoS Genetics*, 9, e1003264

FIGURE LEGENDS

Figure 1. Map of *L. dispar* spread rates along the southern invasion front of Virginia and West Virginia in 10 year increments (1995, 2005, 2015) courtesy of Laura Blackburn (USDA Forest Service). Localities of the source populations and experimental sites within the reciprocal transplant are indicated.

Figure 2. Thermal regimes for reciprocal transplant and controlled chamber experiment by number of hours subjected to designated grouping. Hours spent < 12°C is considered to be suboptimal, 26-28°C is considered optimal, and > 28°C is considered supraoptimal with each increasing °C to be exponentially detrimental. The end date for hourly calculation was based on time at 95% adult emergence within each experimental environment. Sub-, optimal, and supraoptimal temperatures were selected based on the prior studies by Logan et al. (1991) and Tobin et al. (2014). (AM: Appalachian Mountains; CP: Coastal Plain; MW: Mountain Warm; CC: Coastal Cool; CW: Coastal Warm; C+: Coastal Warm +1.7°C).

Figure 3. Phenotypic measurements are represented by mean and standard error for the reciprocal transplant experiment. Phenotypes differentiate between environmental location but not by population. (a) Pupal mass is measured in grams. (b) Larval development time is measured by time from hatch to pupal formation in days. (c) Pupal duration is measured by time from pupal sclerotization to adult emergence in days. Populations are indicated by various point shape and color.

Figure 4. Phenotypic measurements are represented by mean and standard error for the controlled chamber experiment. Similar to that of the reciprocal transplant experiment,

phenotypes differentiate between environmental location but not by population. (a) Pupal mass is measured in grams. (b) Larval development time is measured by time from hatch to pupal formation in days. (c) Pupal duration is measured by time from pupal sclerotization to adult emergence in days. Populations are indicated by various point shape and color.

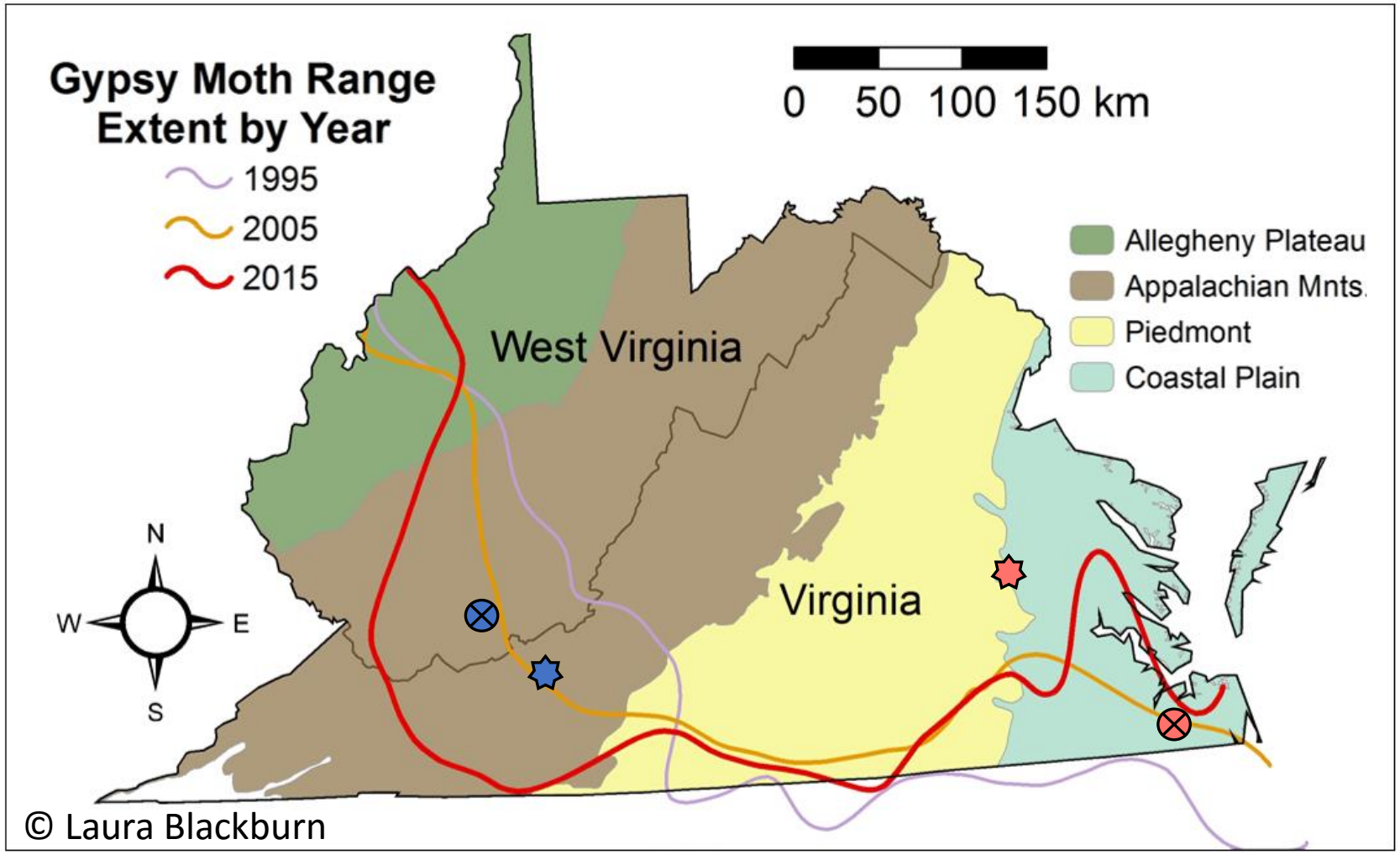
Figure 5. Pie chart representing the percentages of sequences of significantly associated from the GWAS share similarity with other lepidopteran species. Function of these hits were pulled and categorized as: gene if they were associated with a gene of known function; unknown if they aligned to a protein or mRNA with no known function or annotation; and N/A is they had no alignment.

Figure 6. Principal components analysis (PCA) for the reciprocal transplant experiment following Patterson *et al.* (2006) displays partitioning between populations with genetic sub-structuring within the Appalachian Mountain population. First two PCs are labeled with percent of the genetic variance explained (PVE). Populations are indicated by point color.

Figure 7. Allele frequency change (**Δ***af*) analyses. (a) Null distribution of randomized test for allele frequency change shows the number of shared loci was greater than that expected by chance. Number of shared loci are indicated by the arrow on figure with a *P*-value.
(b) Contingency table along with resulting *Chi*-squared analyses determining that the directionality of allele frequency change stays consistent across reciprocal transplant and controlled chamber experiment. Positive indicates the more ambient environment had higher

allele frequency (AM or 26°C) while the negative indicates that the more selective environment had a higher allele frequency (CP, MW, CC, CW, C+).

Figure 1. Map of *L. dispar* spread across the southern invasive range



Appalachian Mountains (AM): ⊗ source population, ✸ experimental site
Coastal Plain (CP): ⊗ source population, ✸ experimental site

Figure 2. Thermal regimes for reciprocal transplant and controlled chamber experiment

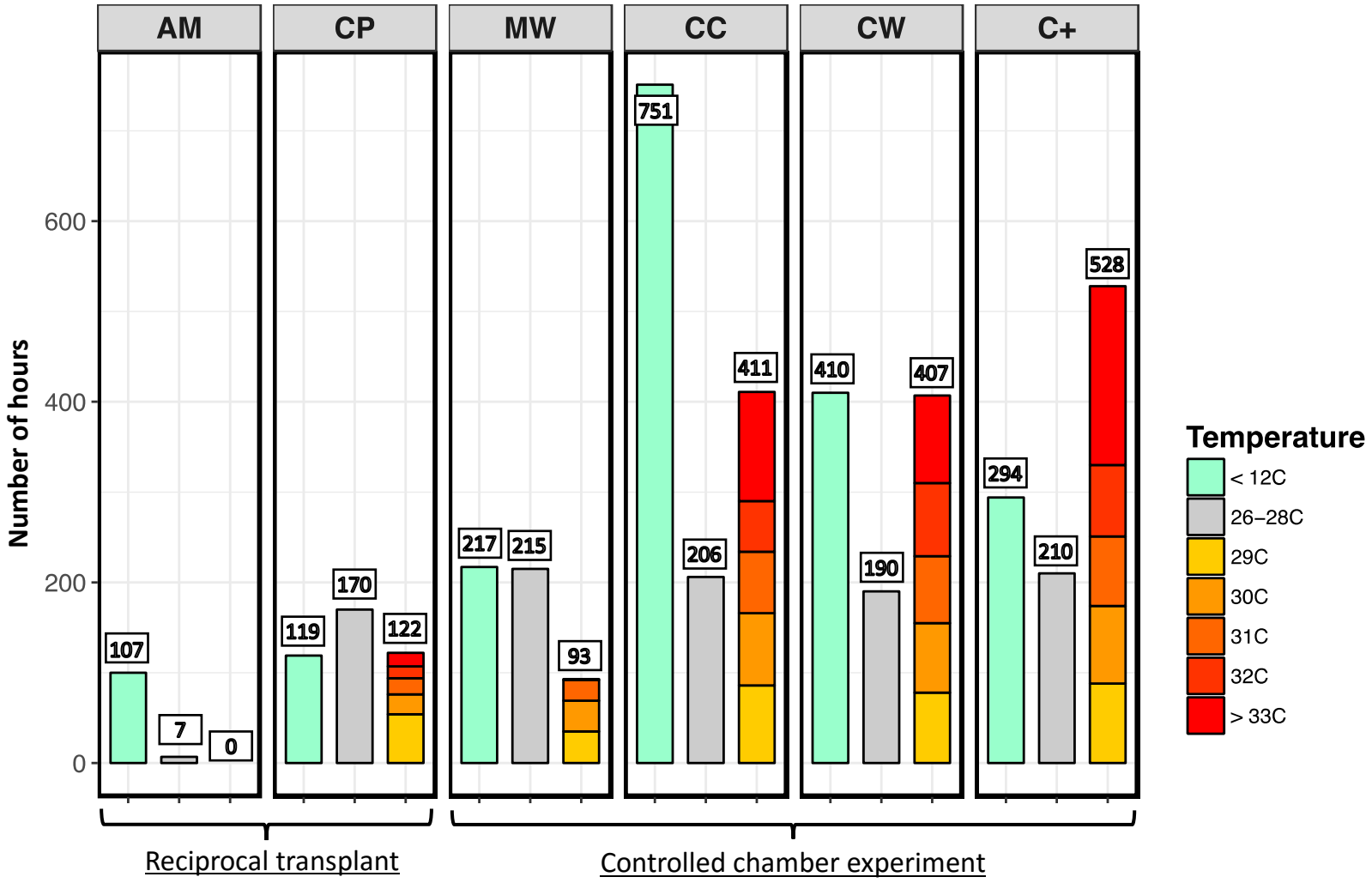


Figure 3. Reciprocal transplant experiment phenotypes

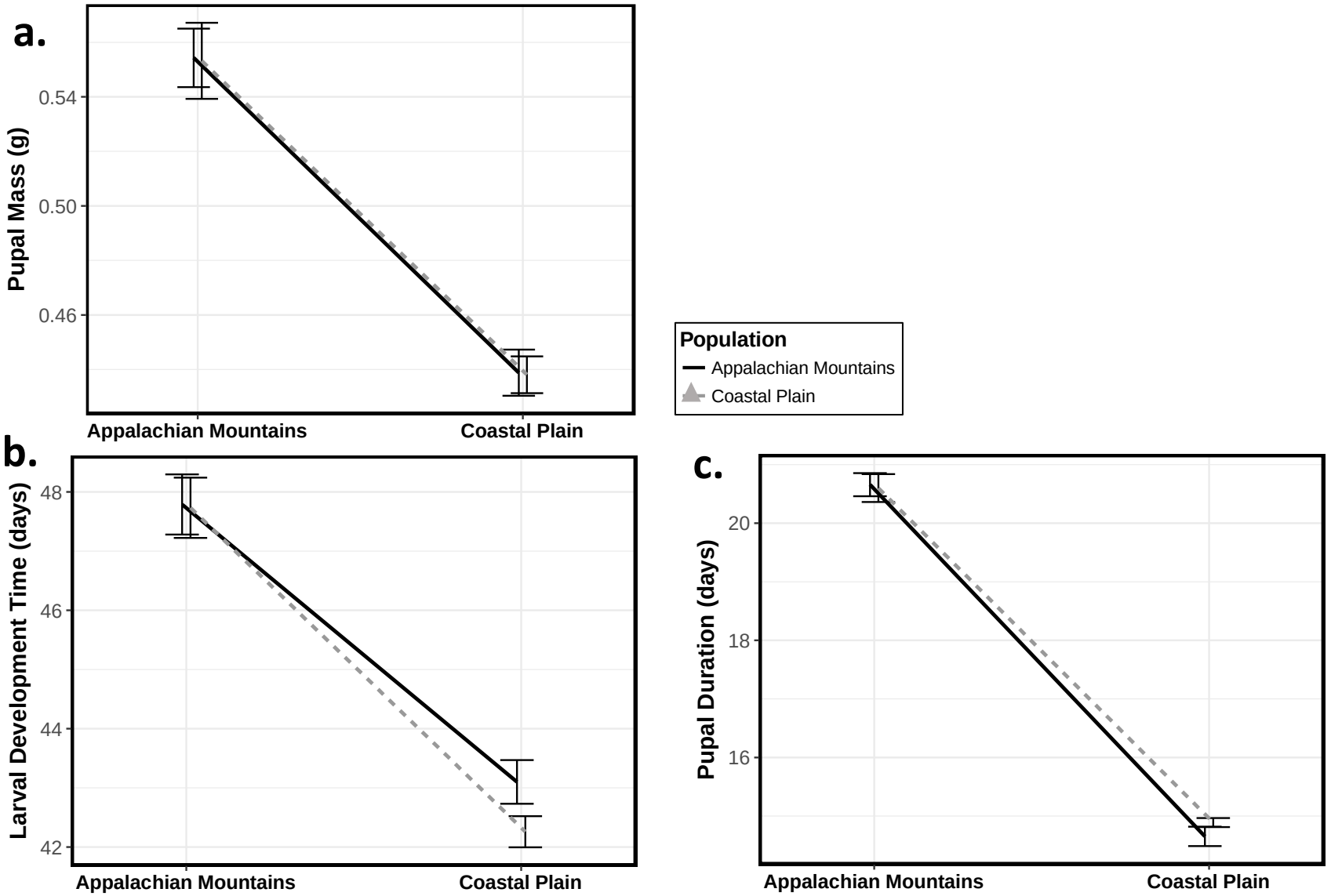


Figure 4. Controlled chamber experiment phenotypes

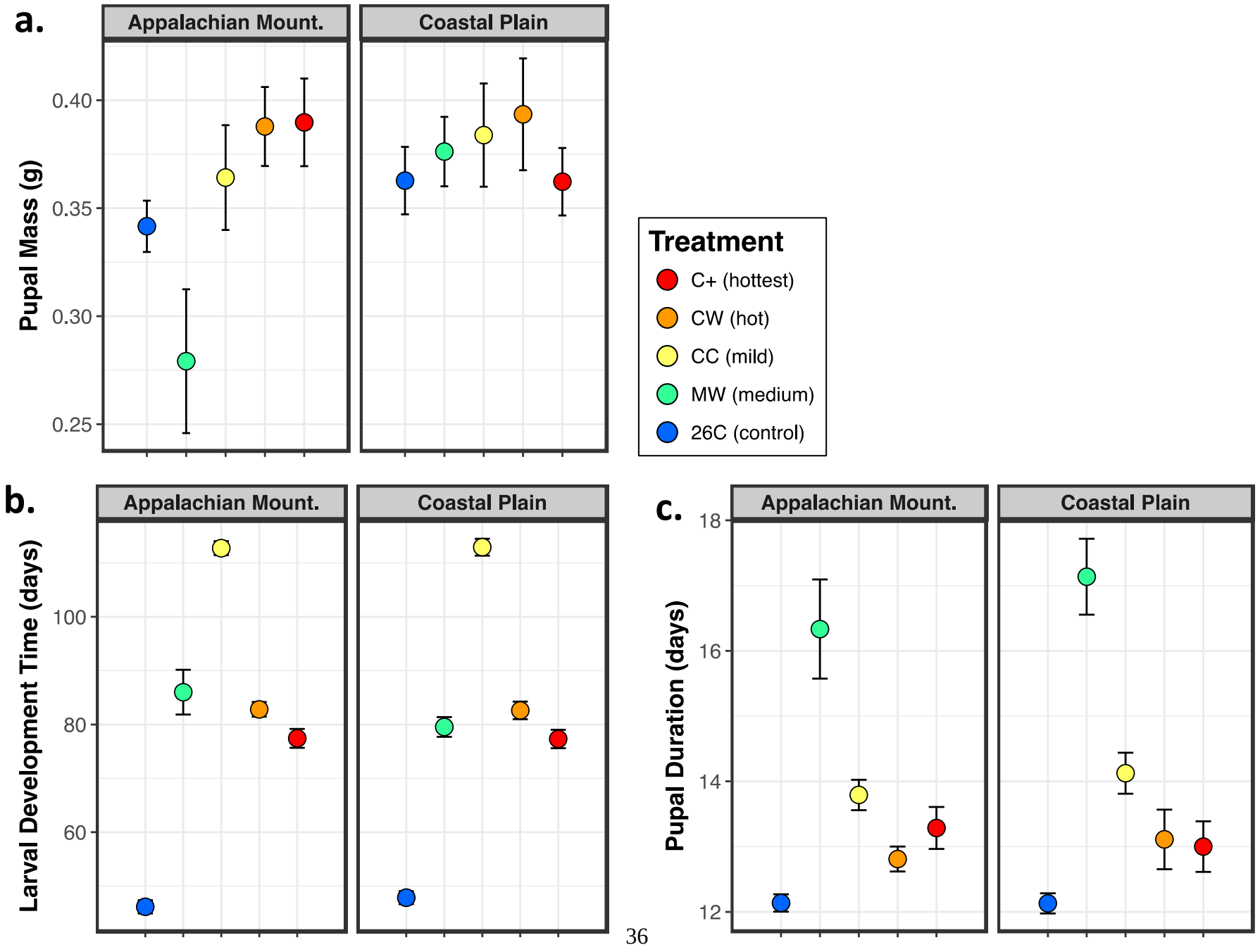


Figure 5. Pie chart of GWAS sequence similarity

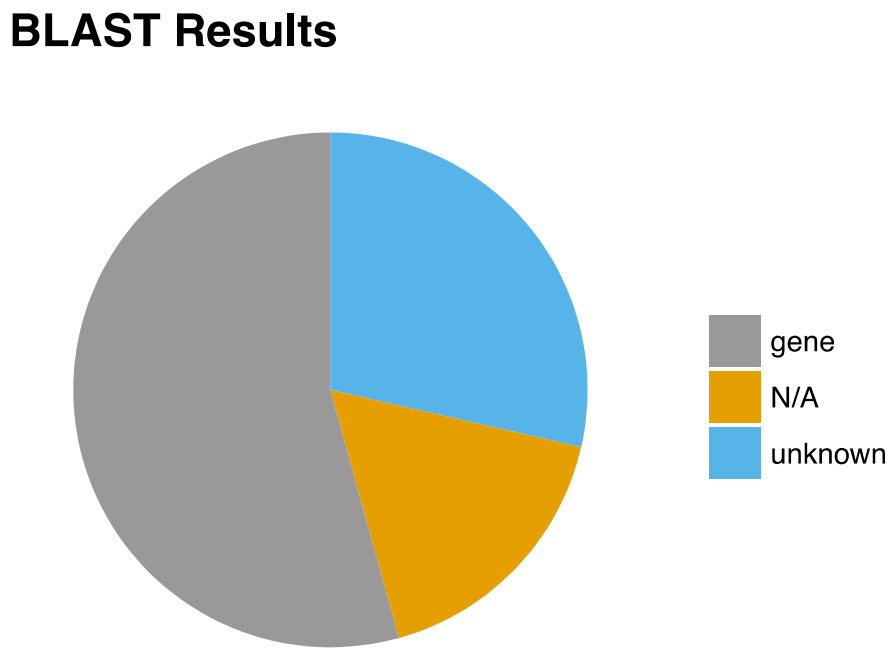


Figure 6. Principal components analysis

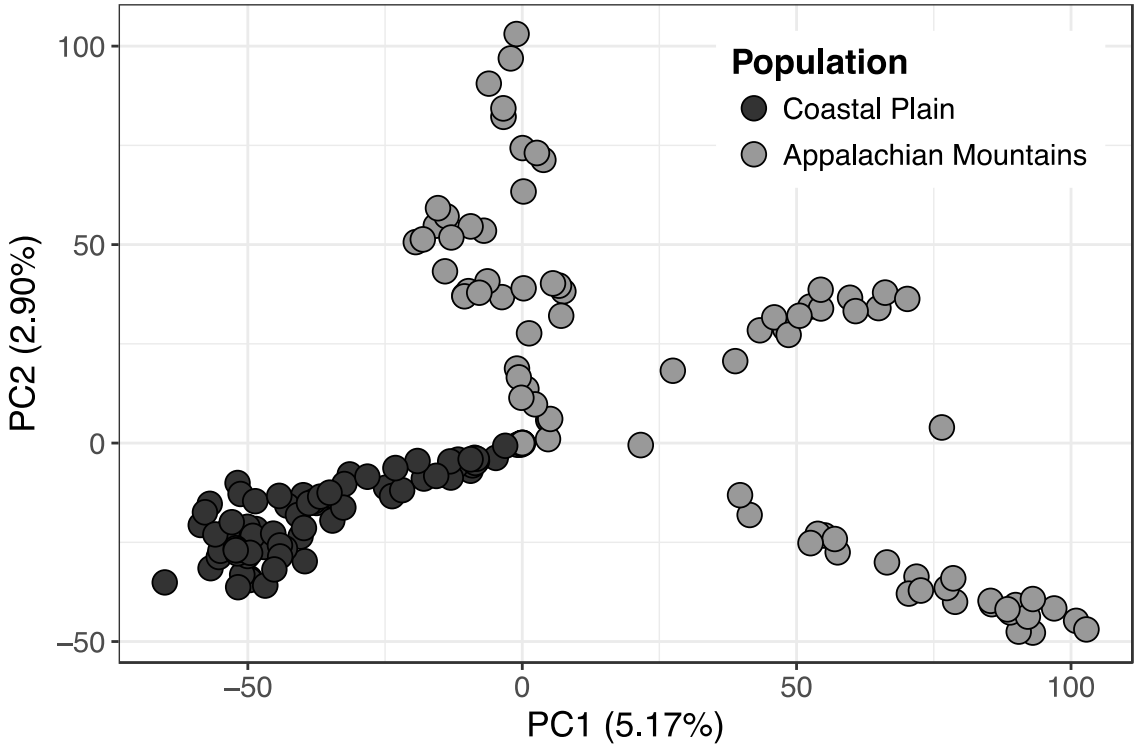
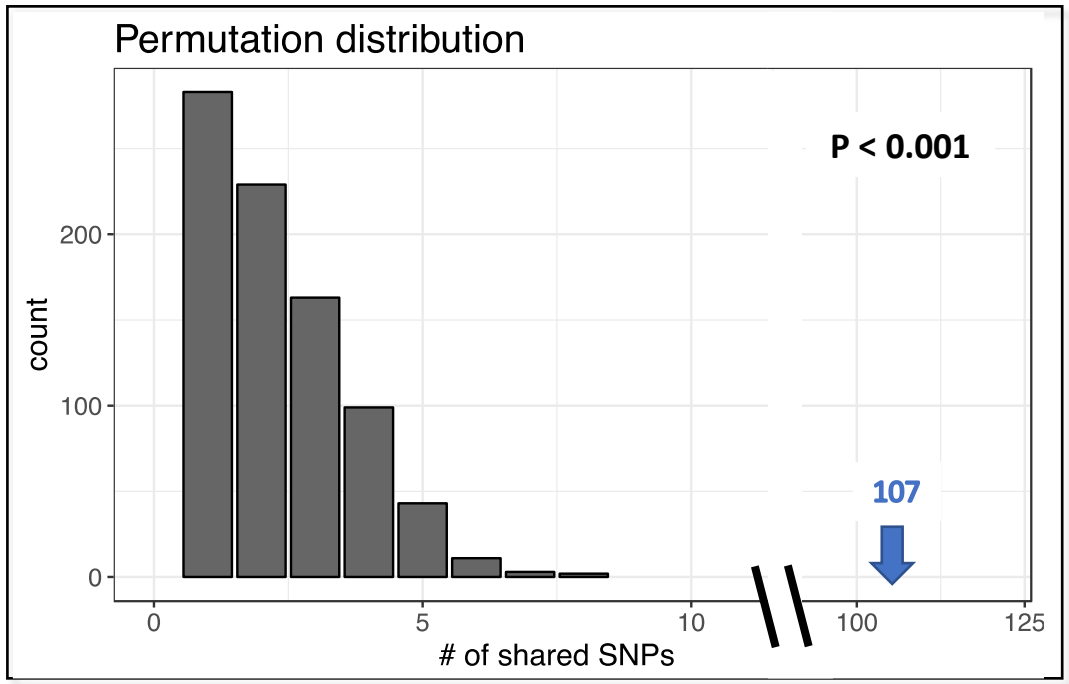


Figure 7. Allele frequency change (**Δ***af*) analyses

a.



b.

	All shared loci		Unique shared loci	
	Positive	Negative	Positive	Negative
Positive	52	42	39	33
Negative	7	6	4	6

Chi-squared	χ^2	*df*	*P*	χ^2	*df*	*P*
	57.99	1	<0.0001	43.85	1	<0.0001

Table 1. Summary of analysis of variance (ANOVA)

Results examine the effect of site/treatment, population, and their interaction on each of the three phenotypes (pupal mass, larval development time, and pupal duration).

	Reciprocal transplant				Controlled chamber experiment			
ANOVA	*MS*	*F*	*df*	*P*	*MS*	*F*	*df*	*P*
Pupal Mass (g)								
Site	0.4968	139.4	1, 167	**<2e-16**	0.0171	2.2329	4, 195	0.0669
Population	0.0002	0.044	1, 160	0.8347	0.0218	2.8385	1, 195	0.0936
Site:Population	0.0001	0.002	1,162	0.9684	0.0116	1.5164	4, 195	0.1989
Larval Dev. Time (d)								
Site	994.7	157.5	1, 167	**<2e-16**	26770	515.53	4, 195	**<2e-16**
Population	8.90	1.410	1, 162	0.2368	38.6	0.74	1, 195	0.3895
Site:Population	6.72	1.064	1, 164	0.3038	60.3	1.16	4, 195	0.3288
Pupal Duration (d)								
Site	1392.5	1248.9	1, 167	**<2e-16**	81.64	38.884	4, 195	**<2e-16**
Population	0.32	0.29	1, 167	0.5922	2.121	1.010	1, 195	0.3161
Site:Population	0.88	0.78	1, 167	0.3769	1.083	0.516	4, 195	0.7243

MS, means squared

Table 2. Summary of genome-wide association study

	LDT	PD
# of sig. loci	32	3
Effect sizes (β)	[-1.807, 0.551]	[-0.515, 0.433]
Effect size (raw)	[-4.715, 1.438]	[-0.547, 0.460]
Q-value	[0.0475, 0.199]	0.121

β: units of standard deviations of normal quantile transformed data
raw: units of raw data (days for both LDT and PD)

Table 3. Summary of overlapping loci in GWAS and **Δ***af* analyses

locus	*Q*-value	beta *(β)*	***Δaf***	phenotype	Top BLAST hit
1	0.0986	-0.448	0.323	LDT	Serine protease
2	0.0850	-0.421	0.317	LDT	*No hit**
3	0.0644	0.483	-0.414	LDT	Vitellogenin gene
4	0.0475	0.505	-0.377	LDT	*Unknown protein*
5	0.1094	-0.353	0.618	LDT	P450 gene
6	0.0986	-0.433	0.300	LDT	Vitellogenin gene
7	0.0749	-0.429	0.449	LDT	*Unknown protein*
8	0.0986	-0.411	0.397	LDT	ABCC gene
9	0.0896	-0.500	0.385	LDT	*Unknown protein*
10	0.1210	-0.473	0.369	PD	LDT1 gene

SUPPLEMENTAL INFORMATION

Table S1. Summaries of trait mean and standard error by experimental environmental site/treatment and population

	Reciprocal transplant		Controlled chamber experiment				
Site / Treatment	Appalachian Mountains	Coastal Plain	Mountain Warm	Coastal Cool	Coastal Warm	Coastal +1.7°C	Control (26°C)
Appalachian Mountains							
Pupal Mass (g)	0.55 (0.011)	0.44 (0.008)	0.28 (0.033)	0.36 (0.024)	0.39 (0.018)	0.39 (0.020)	0.34 (0.012)
Larval Dev. Time (d)	47.8 (0.508)	43.1 (0.369)	86.0 (4.155)	112.8 (1.297)	82.8 (1.357)	77.4 (1.759)	46.1 (1.233)
Pupal Duration(d)	20.66 (0.197)	14.65 (0.166)	16.33 (0.760)	13.79 (0.233)	12.81 (0.190)	13.29 (0.321)	12.14 (0.132)
Sample Size	38	49	6	24	21	14	44
Coastal Plain							
Pupal Mass (g)	0.55 (0.014)	0.44 (0.007)	0.38 (0.016)	0.38 (0.024)	0.39 (0.026)	0.36 (0.016)	0.36 (0.016)
Larval Dev. Time (d)	47.7 (0.509)	42.3 (0.263)	79.5 (1.833)	112.9 (1.569)	82.6 (1.637)	77.3 (1.710)	47.8 (1.206)
Pupal Duration(d)	20.6 (0.237)	14.9 (0078)	17.1 (0.582)	14.1 (0.315)	13.1 (0.457)	13.0 (0. 387)	12.1 (0.154)
Sample Size	30	54	22	16	18	16	24

Figure S1. Histogram of per locus F_{ST} estimate.

Red, dotted-line indicates the mean global multilocus F_{ST}